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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER
PAPPU, SITA S

ART UNIT	PAPER NUMBER
1636	8

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 09/834,778	Applicant(s) SILVER ET AL.	
	Examiner Sita Pappu	Art Unit 1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *04 February 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-50* is/are pending in the application.

4a) Of the above claim(s) *22-50* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-21* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☒ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

Applicants' election of Group I, claims 1-21, is acknowledged. Claims 22-50 are withdrawn from consideration as drawn to non-elected subject matter. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

This paper contains an examination of claims 1-21 on their merits.

Drawings

Drawings are objected to by the draftsperson. See attached PTO-948.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-21 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a nucleic acid molecule comprising a sequence encoding Cre recombinase and a lox P signal site recognized by the said recombinase and/or a nucleic acid molecule comprising at least a first loxP signal site and a second lox P signal site and the cre recombinase gene operably linked to an expression control sequence, such that excision of a sufficient portion of either the recombinase gene or the expression control sequence occurs when the signal sites are contacted with the Cre recombinase, wherein either or both the nucleic acid molecules are included in a retroviral vector and the loxP site is inserted into the said retroviral long terminal repeat

and a cell comprising the retroviral vector, wherein the vector and/or the cell are used for in vitro methods of studying site specific recombination, does not reasonably provide enablement for the use of the said retroviral vector and/or the cell for therapeutic purposes in in vivo controlled delivery of diagnostic and therapeutic agents. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims.

There are many factors to be considered when determining whether there is sufficient evidence to support a determination that a disclosure does not satisfy the enablement requirement and whether any necessary experimentation is "undue." These factors include, but are not limited to: (a) the breadth of the claims; (b) the nature of the invention; (c) the state of the prior art; (d) the relative skill of those in the art; (e) the level of predictability in the art; (f) the amount of direction provided by the inventor; (g) the existence of working examples; and (h) whether the quantity of experimentation needed to make or use the invention based on the content of the disclosure is "undue" (MPEP 2164.01 (a)).

Nature of the Invention:

Claims 1-21 are directed to a retroviral vector comprising a nucleic acid molecule comprising a sequence encoding Cre recombinase and a lox P signal site recognized by the said recombinase and/or a nucleic acid molecule comprising at least a first loxP signal site and a second lox P signal site and the cre recombinase gene operably linked to an expression control sequence, such that excision of a sufficient portion of either the

recombinase gene or the expression control sequence occurs when the signal sites are contacted with the Cre recombinase, wherein either or both the nucleic acid molecules are included in a retroviral vector and the loxP site is inserted ionto the said retroviral long terminal repeat and a cell comprising the retroviral vector, wherein the vector and/or the cell are used for in vitro methods of studying site specific recombination. Further, the invention of the claims 1-21 is intended to be used for the purpose of gene therapy in mammals and/or humans. Therefore, the nature of the invention is directed toward gene therapy using a retroviral vector comprising a cre recombinase gene and loxP sites and expressing a heterologous, therapeutic nucleic acid in cells of mammals and humans after transduction with the said retroviral vector into cells.

Breadth of the Claims:

The claims encompass retroviral vectors comprising a cre recombinase gene and loxP sites, further comprising a heterologous nucleic acid and is used to transduce mammals and/or humans for the purpose of gene thearpy. The claims encompass gene therapy, because one of the purposes of the retroviral vector comprising heterologous nucleic acid, as disclosed by the specification, is for therapeutic purposes (specification, page 3, lines 1-3). Therefore, the claims have a very broad scope, and are not limited to the retroviral vector comprising heterologous nucleic acids and a cell comprising the retroviral vector under in vitro conditions. The specification does not disclose any other purpose or utility for transducing the mammalian and/or human cells and for expressing heterologous nucleic acids encoded on the retroviral vector of the instant invention.

Thus, the claims encompass the application of the said retroviral vector to the whole organism for the purpose of gene therapy and have a very broad scope.

Amount of Direction provided and existence of working examples:

The specification contemplates the use of the retroviral vector of the instant invention for therapeutic purposes (page 3, lines 1-3) and for the production of therapeutic proteins in the milk of transgenic animals (page 3, lines 5-6). Other than this, the specification fails to provide any guidance on using the retroviral vector of the instant invention in gene therapy. The prior art teaches a method of transducing cells and expressing heterologous nucleic acids in cells through the use of retroviral vectors. However, prior art does not teach expression of heterologous nucleic acids for the purpose of gene therapy in mammals and humans to such levels that a therapeutic effect is obtained. In cases where prior art does not teach how to use the method, all the guidance for practicing the invention must come from the specification. The specification fails to disclose how long the enhanced expression of exogenous nucleic acids in the cells of mammals and/or humans lasts, and whether it is long enough to see a therapeutic effect. The working examples do not provide sufficient guidance on how the effect of expression of the heterologous nucleic acids on treating the patient was measured and/or quantified, such that one of skill in the art would accept that their method would result in a therapeutic outcome and be able to practice the method using the guidance provided in the specification. This aspect is particularly relevant since the purpose of the present invention is to provide a method of improved gene delivery and expression for therapeutic purposes.

The specification does not provide guidance to overcome the art recognized unpredictabilities of gene therapy because it lacks correlative evidence between the delivery and expression of a gene and any therapeutic effect. While the specification demonstrates the transduction of cell lines in vitro using the method of the instant invention (Figure 3), it is not predictable that the results obtained in vitro correlate to results expected in vivo such that one of skill would have reasonable expectation of obtaining therapeutic levels of expression of any gene of interest. It is unpredictable how long the enhanced expression of the gene of interest would last such that a therapeutic effect is seen using the method of the instant invention. It would require undue experimentation on the part of a skilled artisan to determine the dosage, frequency and route of administration, to obtain a level of expression that would result in a therapeutic effect.

State of the art:

At the time of filing, *in vivo* gene therapy utilizing the direct administration of recombinant nucleic acids, whether in the form of retroviruses, adenoviruses, or plasmid DNA/liposome complexes, was considered to be highly unpredictable. Verma et al. states that, "[t]he Achilles heel of gene therapy is gene delivery..", and that, "most of the approaches suffer from poor efficiency of delivery and transient expression of the gene" (Verma et al. (1997) Science, Vol. 389, page 239, column 3, paragraph 2). Marshall concurs, stating that, " difficulties in getting genes transferred efficiently to target cells- and getting them expressed- remain a nagging problem for the entire field", and that, "many problems must be solved before gene therapy will be useful for more than the

rare application" (Marshall (1995) Science, Vol. 269, page 1054, column 3, paragraph 2, and page 1055, column 1).

Orkin et al. further states in a report to the NIH that, " .. none of the available vector systems is entirely satisfactory, and many of the perceived advantages of vector systems have not been experimentally validated", and that," [w]hile the expectations and the promise of gene therapy are great, clinical efficacy has not been definitively demonstrated at this time in any gene therapy protocol" (Orkin et al. (1995) "Report and recommendations of the panel to assess the NIH investment in research on gene therapy", page 1, paragraph 3, and page 8, paragraph 2).

Among the many factors that the art teaches affect efficient gene delivery and sustained gene expression are anti-viral immune responses, particularly against adenoviral proteins, and the identity of the promoter used to drive gene expression. Verma et al. teaches that weak promoters produce only low levels of protein, and that only by using appropriate enhancer-promoter combinations can sustained levels of therapeutically effective protein expression be achieved (Verma et al., *supra*, page 240, column 2). Verma et al. further warns that, " .. the search for such combinations is a case of trial and error for a given type of cell" (Verma et al., *supra*, page 240, bridging sentence of columns 2-3). The state of the art is such that no correlation exists between successful expression of a gene and a therapeutic result (Ross et al. Human gene Therapy, vol. 7, pages 1781-1790, September 1996, see page 1789, column 1, first paragraph).

Further, Cannon et al. (Gene Therapy, 2000, pp1-16) state that the currently preferred use of stable producer cell lines precludes the use of cytotoxic components that include the therapeutic gene product itself (page 5, left column, line 6). Cannon et al. (2000) further point out several problems in developing retroviral vectors that are clinically effective (page 7, bridging paragraph of columns and the following paragraph) that include the limited capacity for insertion of foreign sequences, stability of engineered vectors, preclusion of the use of the intron-containing sequences, and finally lack of cost-effective ways to manufacture the vectors at high enough titres with appropriate assurances of safety. Cannon et al. (2000) further point out (page 9, subsection on sustaining and regulating gene expression) that sustained expression of genes is a problem because of suppression by host mechanisms and loss of viability of transduced cell since the body can recognize as foreign a therapeutic gene product and can mount an immune response that will eventually eliminate gene-engineered cells. Finally, Cannon et al. (2000) state that at present, there are no clinical examples of in vivo somatic cell gene therapy (page 13, left column, subsection on 'Clinical Trials: A. Strategies for gene delivery in clinical applications).

Thus, the art at the time of filing clearly establishes that expectation for achieving a desired therapeutic effect *in vivo* by expressing a therapeutic gene using any of the expression constructs known in the art at the time of filing was extremely low.

Predictability of the Art, Amount of Experimentation and Skill level of the artisan:

While it is relatively routine in the gene transfer art to achieve expression at non therapeutic levels, i.e., expression at low levels or at levels providing no patentably

useful phenotypic effect, it is unpredictable without specific guidance and direction whether one will definitively achieve expression of a particular molecule at levels sufficient for a therapeutic effect. Thus, when there is deficiency in the art in terms of predictability of obtaining therapeutic levels of expression, the Applicant must provide sufficient guidance and direction which demonstrates or reasonably correlates to therapeutic levels of expression of a DNA product in an art recognized animal model or patient as claimed.

Even though the skill of an artisan in this subject area is considered to be very high, it would require undue experimentation on the part of an artisan to make and use the invention as specified and use the invention as claimed. The specification and the working examples do not provide sufficient guidance to practice the invention as claimed. Therefore, in the absence of specific guidance and working examples, the use of the claimed retroviral vector comprising heterologous coding sequence encoding a therapeutic polypeptide is unpredictable. In such a situation, one skilled in the art would not know how to use the invention as claimed, without undue experimentation. In view of the limited guidance in the specification, and limited working examples, and the unpredictability of the art, one skilled in the art would be required to engage in undue experimentation, in order to use the invention. It is noted that the law requires that the disclosure of an application shall inform those skilled in the art how to use applicants' alleged discovery, not how to find out how to use it for themselves (see In re Gardner et al. 166 USPQ 138 (CCPA 1970). The specification only teaches what is intended to be done, but does not actually teach how to do that which is intended.

Thus, due to the art recognized unpredictability of achieving therapeutic levels of gene expression following direct or indirect administration of retroviral vectors, the lack of guidance provided by the specification for the parameters affecting delivery and expression of therapeutic amounts of DNA into the cells using the retroviral vector of the instant invention, the lack of guidance concerning the treatment of various diseases using the claimed heterologous coding sequences encoding a therapeutic polypeptide of the instant invention, it would have required undue experimentation to practice the instant invention and the skilled artisan would not have predicted success in using the claimed retroviral vector in methods of transduction and expression for the purpose of gene therapy as disclosed in the specification. Thus the specification does not enable one skilled in the art to use the claimed invention in gene therapy.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 1-21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sauer (1996, Nucleic acids research, vol. 24, no.23, pp.4608-4613) and Gagneten et al. (1997, Nucleic acids research, vol. 25, no.16, pp.3326-3331), further in view of Miyoshi et al. (1998, Journal of virology, vol. 72, no.10, pp.8150-8157).

Sauer (1996) states that Cre/lox system is used to efficiently excise DNA from genome by site-specific recombination at the loxP sites catalyzed by the Cre recombinase (page 4608, left column, 'Introduction', lines 1-15) in vitro and also in mice.

Sauer (1996) further states that recombination between the chromosomal loxP sites by Cre recombinase will generate complicating and unwanted chromosomal translocations, deletions and/or inversions (page 4613, left column, lines 2-5).

Sauer does not teach the self excising Cre of the instant invention.

Gagneten et al. (1997, Nucleic acids research, vol. 25, no.16, pp.3326-3331) teach by utilizing a Cre-GFP fusion, (page 3328, left column, lines 1-6) that transient expression of Cre recombinase efficiently evicts loxP-flanked DNA from mammalian genome (page 3328, right column, lines 8-13) and that a brief burst of Cre expression commits a transfected cell to Cre-mediated excision of DNA (page 3329, right column, paragraph 3, lines 9-12) and that the recombination potential of a transfected cell correlates with the level of Cre expression (page 3330, right column, lines 17-18).

Gagneten et al. Do not teach the transient expression of Cre by self excision.

Miyoshi et al. (1998, Journal of virology, vol. 72, no.10, pp.8150-8157) teach that self-inactivating (SIN) lentiviral vectors constructed by deleting the U3 region of the 3' LTR are safer because of the transcriptional inactivation of the LTR in proviruses.

Miyoshi et al. Do not teach their SIN vectors in the context of Cre/lox system.

The SIN lentiviral vectors of Miyoshi et al. successfully demonstrated that deleting the 3' LTR sequences is an efficient way to improve safety of retroviral vectors. Therefore, it would have been obvious to one one of ordinary skill in the art to be motivated to use the Cre/lox system to delete the Cre recombinase gene itself, with a reasonable expectation of success, to inactivate further recombination that might lead to genomic instability and toxicity, once the vector transfected the cell and expressed the Cre recombinase, following the example of SIN lentiviral vectors, in which case it was successfully demonstrated that SIN lentiviral vectors improve safety of using retroviral vectors.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Sita S Pappu whose telephone number is (703) 305-5039. The examiner can normally be reached on Mon-Fri (8:30 AM - 5:00 PM).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Irem Yucel can be reached on (703) 305 1998. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 746 7442 for regular communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 305-2982.

Anne-Marie Baker

ANNE-MARIE BAKER
PATENT EXAMINER

S. Pappu
March 15, 2002